# Myocardial and systemic carbohydrate metabolism in lambs with aortopulmonary left-to-right shunts at rest and during exercise

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Congenital heart disease with left-to-right shunts - such as a ventricular septal defect or a patent ductus arteriosus - leads to an increase in pulmonary blood flow and a volume load for the left atrium and the left ventricle of the heart. The increased volume load for the heart leads to increased cardiac work and as an adaptation hypertrophy will occur. To cope with the higher work load, the hypertrophied heart has to consume more fuel and oxygen.

Under normal conditions free fatty acids (FFA) are the major source of energy for the myocardium. Other substrates such as carbohydrates, lactate and ketone bodies can also serve as fuel for the myocardium, especially under conditions of increased supply of these substrates. Cardiac muscle can shift from one source of energy to another according to the supply. For example, during a bout of exercise, in which the arterial concentration of lactate increases, the myocardium prefers lactate to FFA. An important feature of exercise is that the heart can utilize lactate discharged by skeletal muscles for immediate energy production, thereby preserving the myocardial glycogen and triglyceride stores for use under circumstances of inadequate exogenous substrate supply. Adrenaline seems to facilitate the use of lactate and glucose by the heart. The same is true for an increase in energy expenditure by raising the mechanical work done. But also during a chronic load the myocardium seems to prefer lactate to FFA, even when the arterial lactate concentration is not increased. In lambs with a chronic volume load due to an aortopulmonary shunt a higher myocardial net lactate uptake was found than in control lambs. The volume-loading of these hearts by the aortopulmonary shunt appears to lead to a shift in myocardial substrate uptake from FFA to carbohydrates, like in pressure-overloaded hearts.

Studying myocardial metabolism by considering only net uptake of substrates by the myocardium gives limited information, because uptake and release of substrates can occur simultaneously. Studies with tracers such as stable and radioactive isotopes have demonstrated that, for example, the myocardial net lactate uptake is the result of both lactate uptake and lactate release. Even in the normal myocardium, lactate has been shown to be taken up and released simultaneously. The higher myocardial net lactate uptake found previously in lambs with an aortopulmonary shunt thus may have been the result of a higher myocardial lactate uptake and/or a decreased myocardial lactate release.

For this study an animal model - lambs with an aortopulmonary shunt - was used. Lambs of mixed breed with documented dates of birth were studied. They were operated at the age of 4 - 5 weeks. In the shunt lambs a Goretex conduit was sutured between the descending aorta and the main pulmonary artery. Catheters were inserted into the aorta, the coronary sinus, the pulmonary artery, the right ventricle (only in the shunt lambs), and the right and left atrium. Precalibrated electromagnetic flow transducers were placed around the ascending aorta and around the pulmonary artery to determine pulmonary and systemic blood flow. Myocardial blood flow was measured with the aid of radioactively labeled microspheres.

The experiments were performed, after an overnight fast of 18h, in conscious lambs at rest and during moderate exercise on a treadmill. Until the day before the

first experiment (2 - 3 weeks after the operation) and between the experiments the lambs remained with their mothers. The lambs were randomly divided into two groups. In one group (8 shunt and 12 control lambs) the experiments were performed at rest with the lamb hanging in a sling; the other group (9 shunt and 10 control lambs) was familiarized with running on a treadmill and was investigated during exercise (50% of $\dot{V}O_{2\ peak}$ for 30 min). The latter group underwent a peak oxygen consumption test ($\dot{V}O_{2\ peak}$ experiment) 1 - 2 weeks after the operation.

In each group each lamb underwent, if possible, in random order two similar studies each with different $^{13}$C-labeled substrates ([1-$^{13}$C]lactate or [U-$^{13}$C]glucose) at two different days with at least 3 days in-between for recovery. The myocardial glucose and lactate oxidation was investigated through determination of $^{13}CO_2$ concentrations in the aorta and coronary sinus. The glucose production rates, gluconeogenesis and glycogenolysis were investigated with the help of [U-$^{13}$C]glucose. The lactate production rates were investigated with the help of [1-$^{13}$C]lactate.

Until now, oxidation of substrates by the heart was studied with the aid of radioactive isotopes. Investigation of myocardial substrate oxidation with the aid of stable isotopes is preferable to radioactive isotopes, because it avoids the hazards and inconveniences of radioactivity. Therefore, in this investigation a method to study oxidation of $^{13}$C-labeled substrates by an organ with the aid of a stable isotope is described and used for the determination of glucose and lactate oxidation by the myocardium. To study oxidation of $^{13}$C-labeled substrates we had to determine $^{13}CO_2$ concentrations in blood, for which a suitable method had not yet been described. Chapter 4 shows that with the combination of two established methods, one for the determination of total $CO_2$ concentration in blood and one for the isotope ratio of $CO_2$, it is possible to measure organ substrate oxidation in vivo by means of $^{13}$C-labeled substrates.

The main object of the study was to determine myocardial glucose and lactate oxidation in lambs with an aortopulmonary shunt, in order to test the hypothesis that, to meet the higher $O_2$ demand, myocardial lactate and glucose uptake and oxidation would be increased in shunt lambs in comparison with control lambs. We found that, after an overnight fast, myocardial lactate oxidation is indeed higher in shunt than in control lambs, despite similar arterial lactate concentrations. During exercise myocardial lactate oxidation is increased in both groups of lambs. There was no difference in myocardial lactate release between shunt and control lambs. Oxidation of exogenous glucose, which was approximately zero at rest, increased during exercise in shunt and control lambs. Thus it appears that a higher contribution of carbohydrates to cardiac metabolism not only occurs in case of pressure-overloaded hearts, but also in myocardial hypertrophy due to volume-overloading (chapter 5).

As to the hypothesis that if the hypertrophied myocardium of lambs with an aortopulmonary shunt oxidizes more lactate, the activities of LDH and PDH would be higher in various compartments of the myocardium of shunt lambs in comparison with control lambs, we found indeed regional differences in activities of total LDH and LDH isoenzymes, and of total PDH and $PDH_a$ in the

hypertrophied myocardium. These results suggest differences in carbohydrate metabolism in some compartments of the heart between shunt and control lambs. We speculate that the higher myocardial lactate oxidation found in lambs with an aortopulmonary shunt predominantly takes place in the left atrium (chapter 6).

Hypoglycemia has been described in children with acute congestive heart failure, mostly due to congenital heart disease. To investigate whether hypoglycemia also occurs in lambs with an aortopulmonary shunt, we determined the blood glucose concentration during and after an overnight fast and measured the glucose production rate, gluconeogenesis and glycogenolysis with the help of [U-$^{13}$C]glucose. We found that after an overnight fast arterial glucose concentrations were lower in lambs with aortopulmonary shunts than in control lambs. This lower glucose concentration was caused by a decreased glucose production rate. Glycogenolysis was decreased in shunt lambs as compared to control lambs, while there was no difference in gluconeogenesis. There was no difference in hormonal control. Therefore, we surmise that the shunt lambs have limited glycogen stores that are more readily depleted than those of the control lambs (chapter 7).

During our studies in shunt and control lambs after an overnight fast, and in previous studies during exercise, it has been demonstrated that shunt lambs had a lower systemic blood flow than control lambs. An impaired systemic oxygen supply may lead to an oxygen deficit in peripheral tissues with a consequent increase in anaerobic metabolism. Therefore, we investigated the lactate production rate in shunt lambs in comparison with control lambs, both at rest and during exercise. We found that lambs with an aortopulmonary shunt have the same lactate kinetics as control lambs at rest and during moderate exercise performed at a similar fraction of their (considerably lower) $\dot{V}O_{2\ peak}$, despite the lower systemic oxygen supply. We speculated that the shunt lambs have adapted themselves to the decreased systemic oxygen supply through consuming less oxygen by limiting the amount of external work (chapter 8).

At rest, we found a lower arterial glucose concentration and a lower glucose production rate in the shunt lambs in comparison with the control lambs and speculated that a depletion of the glycogen stores in the shunt lambs may be responsible. During moderate exercise the arterial glucose concentration and the glucose production rate normally increase. If the glycogen stores are indeed depleted, the shunt lambs would not be able to increase the arterial glucose concentration and the glucose production rate during moderate exercise. We found that shunt lambs have lower arterial glucose concentrations than control lambs, both at rest and during moderate exercise. This was due to a lower glucose production rate, in particular a lower glycogenolysis. In both shunt and control lambs an increase in the arterial concentrations of pyruvate, lactate, FFA, and free and total glycerol occurred during exercise. We found no difference in catecholamines between shunt and control lambs. Therefore, we presume that catecholamines are not responsible for the differences in glucose kinetics between shunt and control lambs (chapter 9).